Application No.: 10/039,760 Office Action Dated: April 27, 2006

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claims 1-32 (Cancelled)

33. (Currently amended) A method for reducing colonization of enterohemorrhagic *Escherichia coli* (EHEC) in a non-human mammal comprising administering to said non-human mammal an effective amount of a composition comprising an <u>isolated</u> EHEC cell culture supernatant, wherein the cell culture supernatant is produced by the process of incubating the cell culture in media comprising minimal media supplemented with 0.1% Casamino Acids, 0.4% glucose, 8 mM MgSO₄ and 44 mM NaHCO₂.

- 34. **(Currently amended)** A method for reducing shedding of enterohemorrhagic *Escherichia coli* (EHEC) from a non-human mammal comprising administering to said non-human mammal an effective amount of a composition comprising an <u>isolated</u> EHEC cell culture supernatant, wherein the cell culture supernatant is produced by the process of incubating the cell culture in media comprising minimal media supplemented with 0.1% Casamino Acids, 0.4% glucose, 8 mM MgSO₄ and 44 mM NaHCO₂.
- 35. **(Previously presented)** The method of claim 33, wherein the non-human mammal is a ruminant.
- 36. **(Previously presented)** The method of claim 35, wherein the ruminant is a bovine subject.

Application No.: 10/039,760 Office Action Dated: April 27, 2006

37. **(Previously presented)** The method of claim 33, wherein the composition further comprises an immunological adjuvant.

- 38. **(Previously presented)** The method of claim 33, wherein the EHEC is EHEC 0157:H7.
- 39. **(Previously presented)** The method of claim 33, wherein the EHEC is EHEC O157:NM.
- 40. **(Previously presented)** The method of claim 37, wherein the immunological adjuvant comprises an oil-in-water emulsion.
- 41. **(Previously presented)** The method of claim 37, wherein the immunological adjuvant comprises a mineral oil and dimethyldioctadecylammonium bromide.
- 42. **(Previously presented)** The method of claim 37, wherein the immunological adjuvant comprises a non-oil-in-water emulsion.
- 43. (Previously presented) The method of claim 37, wherein the immunological adjuvant is present in the composition at a concentration of 20% to 40% (v/v).
- 44. (Previously presented) The method of claim 43, wherein the immunological adjuvant is present in the composition at a concentration of 30% (v/v).
- 45. **(Previously presented)** The method of claim 33, wherein the composition further comprises one or more recombinant or purified EHEC antigens selected from the group consisting of EspA, EspB, EspD, Tir and Intimin.

Application No.: 10/039,760 Office Action Dated: April 27, 2006

46. **(Currently amended)** The method of claim 45, wherein EspA+Tir comprise 10% to 50% of the cell protein cell culture supernatant present in the composition.

- 47. **(Previously presented)** The method of claim 37, wherein the composition further comprises one or more recombinant or purified EHEC antigens selected from the group consisting of EspA, EspB, EspD, Tir and Intimin.
- 48. **(Currently amended)** The method of claim 47, wherein EspA+Tir comprise 10% to 50% of the eell protein cell culture supernatant present in the composition.
- 49. **(Previously presented)** The method of claim 34, wherein the non-human mammal is a ruminant.
- 50. **(Previously presented)** The method of claim 49, wherein the ruminant is a bovine subject.
- 51. **(Previously presented)** The method of claim 34, wherein the composition further comprises an immunological adjuvant.
- 52. **(Previously presented)** The method of claim 34, wherein the EHEC is EHEC 0157:H7.
- 53. (Previously presented) The method of claim 34, wherein the EHEC is EHEC 0157:NM.
- 54. **(Previously presented)** The method of claim 51, wherein the immunological adjuvant comprises an oil-in-water emulsion.

Application No.: 10/039,760
Office Action Dated: April 27, 2006

56. (Previously presented) The method of claim 51, wherein the immunological adjuvant comprises a non-oil-in-water emulsion.

- 57. (Previously presented) The method of claim 51, wherein the immunological adjuvant is present in the composition at a concentration of 20% to 40% (v/v).
- 58. (Previously presented) The method of claim 51, wherein the immunological adjuvant is present in the composition at a concentration of 30% (v/v).
- 59. (Previously presented) The method of claim 34, wherein the composition further comprises one or more recombinant or purified EHEC antigens selected from the group consisting of EspA, EspB, EspD, Tir and Intimin.
- 60. (Currently amended) The method of claim 59, wherein EspA+Tir comprise 10% to 50% of eell protein the cell culture supernatant present in the composition.
- 61. **(Previously presented)** The method of claim 51, wherein the composition further comprises one or more recombinant or purified EHEC antigens selected from the group consisting of EspA, EspB, EspD, Tir and Intimin.
- 62. (Currently amended) The method of claim 61, wherein EspA+Tir comprise 10% to 50% of the cell culture supernatant present in the composition.
- 63. (Currently amended) The method of claim 37[[or 51]], wherein the immunological adjuvant comprises an agent selected from the group consisting of an emulsifying agent, a muramyl dipeptide, an aqueous agent, a chitosan-based agent, a saponin,

Application No.: 10/039,760
Office Action Dated: April 27, 2006

an oil, a lipopolysaccharide, a bacterial cell wall extract, a bacterial DNA, a bacterial

complex, a synthetic oligonucleotide, and a aliphatic nitrogenous base.

64. **(Previously presented)** The method of claim 63, wherein the

emulsifying agent is selected from the group consisting of a natural emulsifying agent, a

synthetic emulsifying agent, an anionic emulsifying agent, a cationic emulsifying agent, and a

nonionic agent.

65. (Previously presented) The method of claim 64, wherein the natural

emulsifying agent is selected from the group consisting of acacia, gelatin, lecithin, and

cholesterol.

66. **(Previously presented)** The method of claim 64, wherein the anionic

emulsifying agent is selected from the group consisting of a potassium salt of lauric acid, a

potassium salt of oleic acid, a sodium salt of lauric acid, a sodium salt of oleic acid, an

ammonium salt of lauric acid, an ammonium salt of oleic acid, a calcium salt of a fatty acid, a

magnesium salt of a fatty acid, an aluminum salt of a fatty acid, a metallic soap, and an

organic sulfonate.

67. **(Previously presented)** The method of claim 66, wherein the organic

sulfonate is sodium lauryl sulfate.

68. (Previously presented) The method of claim 64, wherein the cationic

emulsifying agent is ceryltrimethylammonium bromide.

Page 7 of 24

PATENT **DOCKET NO.:** UBCV-0006/01-010

Application No.: 10/039,760

Office Action Dated: April 27, 2006

69. The method of claim 64, wherein the synthetic (Currently amended) [[nonionic]]agent is selected from the group consisting of a glyceryl ester, a polyoxyethylene glycol ester, a polyoxyethylene glycol ether, and a sorbitan fatty acid ester.

- 70. (Previously presented) The method of claim 69, wherein the glyceryl ester is glyceryl monostearate.
- 71. (Previously presented) The method of claim 69, wherein the sorbitan fatty acid ester is selected from the group consisting of a sorbitan monopalmitate and polyoxyethylene derivatives thereof.
- 72. (Previously presented) The method of claim 69, wherein the polyoxyethylene derivatives is polyoxyethylene sorbitan monopalmitate.
- 73. (Previously presented) The method of claim 63, wherein the aqueous agent is aluminum hydroxide.
- 74. (Previously presented) The method of claim 63, wherein the oil is selected from the group consisting of a mineral oil, a vegetable oil, and an animal oil.
- 75. (Previously presented) The method of claim 74, wherein the vegetable oil is selected from the group consisting of canola oil, almond oil, cottonseed oil, corn oil, olive oil, peanut oil, safflower oil, sesame oil, and soybean oil.
- 76. The method of claim 74, wherein the animal oil (Previously presented) is selected from the group consisting of cod liver oil, halibut oil, menhaden oil, orange roughy oil and shark liver oil.

Application No.: 10/039,760
Office Action Dated: April 27, 2006

77. (Currently amended) The method of claim 37[[or 51]], wherein the immunological adjuvant comprises an oil component.

78. **(Previously presented)** The method of claim 77, wherein the oil component is selected from the group consisting of a single oil, and a mixture of oils.

79-82. (Cancelled)

- 83. (Currently amended) The method of claim 37[[or 51]], wherein the immunological adjuvant comprises Mycobacterial cell wall extract.
- 84. (Currently amended) The method of claim 37[[or 51]], wherein the immunological adjuvant comprises Mycobacterial DNA.
- 85. (Currently amended) The method of claim 37[[or 51]], wherein the immunological adjuvant comprises a Mycobacterial cell wall complex.
- 86. (**Previously presented**) The method of claim 63, wherein the aliphatic nitrogenous base is selected from the group consisting of an amine, a quaternary ammonium compound, a guanidine, a benzamidine, and a thiouronium.
- 87. (Currently amended) The method of claim 37[[or 51]], wherein the immunological adjuvant comprises dimethyl-dioctade cylamrnonium bromide.
- 88. **(Previously presented)** The method of claim 63, wherein the aliphatic nitrogenous base is N,N-dioctadecyl-N,N-bis(2-hydroxyethyl)propanediamine.

DOCKET NO.: UBCV-0006/ 01-010

PATENT

Application No.: 10/039,760 **Office Action Dated:** April 27, 2006

89. **(Currently amended)** The method of claim 46, [[48, 60, or 62,]] wherein EspA+Tir comprise 20% of the cell protein culture supernatant present in the composition.

- 90. (Currently amended) The method of claim 35[[or 49]], wherein the ruminant is an ovine subject.
- 91. **(New)** The method of claim 51, wherein the immunological adjuvant comprises an agent selected from the group consisting of an emulsifying agent, a muramyl dipeptide, an aqueous agent, a chitosan-based agent, a saponin, an oil, a lipopolysaccharide, a bacterial cell wall extract, a bacterial DNA, a bacterial complex, a synthetic oligonucleotide, and a aliphatic nitrogenous base.
- 92. **(New)** The method of claim 51, wherein the immunological adjuvant comprises an oil component.
- 93. **(New)** The method of claim 56, wherein the non-oil-in-water emulsion is selected from the group consisting of an oil emulsion, a water-in-oil emulsion, and a water-in-oil-in-water emulsion.
- 94. **(New)** The method of claim 51, wherein the immunological adjuvant comprises Mycobacterial cell wall extract.
- 95. **(New)** The method of claim 51, wherein the immunological adjuvant comprises Mycobacterial DNA.

Application No.: 10/039,760
Office Action Dated: April 27, 2006

Office Action Dates. April 27, 2000

96. **(New)** The method of claim 51, wherein the immunological adjuvant comprises a Mycobacterial cell wall complex.

- 97. **(New)** The method of claim 51, wherein the immunological adjuvant comprises Mycobacterial cell wall extract.
- 98. **(New)** The method of claim 51, wherein the immunological adjuvant comprises Mycobacterial DNA.
- 99. **(New)** The method of claim 51, wherein the immunological adjuvant comprises a Mycobacterial cell wall complex.
- 100. **(New)** The method of claim 51, wherein the immunological adjuvant comprises dimethyl-dioctade cylammonium bromide.
- 101. **(New)** The method of claim 48, wherein EspA+Tir comprise 20% of the cell protein culture supernatant present in the composition.
- 102. **(New)** The method of claim 60, wherein EspA+Tir comprise 20% of the cell protein culture supernatant present in the composition.
- 103. **(New)** The method of claim 62, wherein EspA+Tir comprise 20% of the cell protein culture supernatant present in the composition.
- 104. **(New)** The method of claim 49, wherein the ruminant is an ovine subject.

Application No.: 10/039,760
Office Action Dated: April 27, 2006

105. **(New)** The method of claim 33, wherein the cell culture supernatant is concentrated.

- 106. **(New)** The method of claim 34, wherein the cell culture supernatant is concentrated.
- 107. **(New)** A method for reducing colonization of enterohemorrhagic *Escherichia coli* (EHEC) in a non-human mammal comprising administering to said non-human mammal an effective amount of a composition comprising an isolated EHEC cell culture supernatant containing one or more EHEC serotypes wherein the cell culture supernatant is produced by the process of incubating the cell culture in media comprising minimal media supplemented with 0.1% Casamino Acids, 0.4% glucose, 8 mM MgSO₄ and 44 mM NaHCO₂.
- 108. **(New)** The method of claim 107, wherein the cell culture supernatant is concentrated.
- 109. **(New)** The method of claim 107, wherein the one of more EHEC serotypes are selected from the group consisting of O157, O158, O5, O8, O18, O26, O45, O48, O52, O55, O75, O76, O78, O84, O91, O103, O104, O111, O113, O114, O116, O118, O119, O121, O125, O28, O145, O146, O163, and O165.
- 110. **(New)** The method of claim 107, wherein the step of administering is by a route selected from the group consisting of oral, topical, subcutaneous, intramuscular, intravenous, subcutaneous, intradermal, transdermal and subdermal.

Application No.: 10/039,760
Office Action Dated: April 27, 2006

111. **(New)** A method for reducing shedding of enterohemorrhagic *Escherichia coli* (EHEC) from a non-human mammal comprising administering to said non-human mammal an effective amount of a composition comprising an isolated EHEC cell culture supernatant containing one or more EHEC serotypes wherein the cell culture supernatant is produced by the process of incubating the cell culture in media comprising minimal media supplemented with 0.1% Casamino Acids, 0.4% glucose, 8 mM MgSO₄ and 44 mM NaHCO₂.

- 112. **(New)** The method of claim 111, wherein the cell culture supernatant is concentrated.
- 113. **(New)** The method of claim 111, wherein the one of more EHEC serotypes are selected from the group consisting of O157, O158, O5, O8, O18, O26, O45, O48, O52, O55, O75, O76, O78, O84, O91, O103, O104, O111, O113, O114, O116, O118, O119, O121, O125, O28, O145, O146, O163, and O165.
- 114. **(New)** The method of claim 111, wherein the step of administering is by a route selected from the group consisting of oral, topical, subcutaneous, intramuscular, intravenous, subcutaneous, intradermal, transdermal and subdermal.
- 115. **(New)** The method of claim 33, wherein the step of administering is by a route selected from the group consisting of oral, topical, subcutaneous, intramuscular, intravenous, subcutaneous, intradermal, transdermal and subdermal.

Application No.: 10/039,760

Office Action Dated: April 27, 2006

116. **(New)** The method of claim 34, wherein the step of administering is by a route selected from the group consisting of oral, topical, subcutaneous, intramuscular, intravenous, subcutaneous, intradermal, transdermal and subdermal.